Remarks

This application has been amended in a manner that is believed to place it in condition for allowance.

Claims 26-68 are pending in the application. Claims 26-49 correspond to previously pending claims 1-4, 11-12, 14-17, and 19-25, respectively.

Independent claim 26 recites a method for investigating a body fluid from a human subject having or suspected of having cancer for disseminated cancer cells. In addition, claim 26 has been amended to recite steps of providing a further cell-containing fraction of the body fluid from the same individual and determining the expression of the genes in the further cell-containing fraction; and comparing the expression for each of the at least 2 genes in the cell-containing fraction with its expression in the further cell-containing fraction. Support for the changes may be found at page 5, lines 29-34; page 7, line 33 – page 8, line 9; page 15, line 10; page 16, line 32; page 17, line 4; page 18, line 26, 37; page 29, line 14-39; page 30, lines 7-18).

Claims 27-42 coincide with claims 2-4, 11-12, 14-17, and 19-25 and have been drafted to avoid the formal matters raised in the outstanding Official Action.

New claims 43-67 are directed to a method for investigating a body fluid from a human subject having or suspected of having cancer for disseminated cancer cells. While the method of claims 26-49 and are directed steps of providing a further cell-containing fraction, wherein the method of claims 50-67 does not comprise such a step.

Claim 68 is directed to a method for investigating a blood or bone marrow

sample from a human subject having or suspected of having cancer for disseminated cancer cells.

Support for new claims 43-68 can be found in the original claims and generally throughout the specification. For example, support for the new claims may be found at page 5, lines 29-34; page 15, line 10; page 16, line 32; page 17, line 4; page 18, line 26; and page 30, lines 7-18.

Claims 1-25 have been canceled without prejudice or disclaimer.

Applicants respectfully submit that no new matter has been added to the disclosure.

In the Final Rejection of January 5, 2009, claims 1, 21 and 23 were objected to for allegedly containing several informalities. Claims 23 and 24 were rejected under 35 U.S.C. 112, second paragraph for allegedly being indefinite. Amendments to the claims were made in the Amendments of April 13, 2009, April 27, 2009, and April 28, 2009 to overcome the objection and under 35 U.S.C. 112, second paragraph. The new claims incorporate these changes to avoid the objection and rejection under 35 U.S.C. 112, second paragraph.

Thus, reconsideration of the objection and rejection under 35 U.S.C. 112, second paragraph are respectfully requested.

Claims 1-4, 6, 11, and 12 and 14-25 were rejected under 35 U.S.C. 112, first paragraph for allegedly not satisfying the enablement requirement. Applicants respectfully submit that claims 26-68 satisfy the enablement requirement for the reason set forth in the present Amendment and Amendment of April 13, 2009.

Indeed, Applicants maintain that by comparing the expression for each of the at least two genes in the cell-containing fraction with its expression in the further cell-containing fraction a comparison is made with the subjects own non-cancer cells (page 29, lines 14-31). Making this comparison is advantageous, but not mandatory. Therefore, claims 50-67 have been added.

According to Claims 50-67, a higher expression of at least one of the genes in the cell-containing fraction as compared to its average expression in subjects not having cancer indicates the presence of disseminated cancer cells in the body fluid. Thus, it is sufficient to determine the expression of the genes in a cell-containing fraction of the body fluid from a human subject having or suspected of having cancer and to compare the expression of the genes with their average expression in subjects not having cancer.

In order to make such comparisons, one skilled in the art has to know the average expression of each of the genes in healthy subjects. This requires determining the expression in a number of healthy subjects (see example 1 of the present specification). However, once the average expression in healthy subjects is known, the claimed method can be carried out without the need of repeating the determination. Therefore, the determination of the average expression in healthy subjects does not form part of the claimed method.

The following is a comparison of the method of claims 47 and 48 with the method defined by method steps (a) to (j) set forth in the final action of January 5, 2009 on pages 4 and 5.

Step (a)

Claim 48 recites a step of obtaining a cell-containing fraction comprising mononuclear cells from blood by passing the body fluid or a cell-containing fraction thereof through a screen with a mesh or pore width of about 20 um and obtaining the cell fraction retained on the screen corresponds to obtaining test fraction C of step (a). Providing a further cell-containing fraction comprising mononuclear cells from the same individual (claim 48) corresponds to obtaining test fraction A of method step (a). However, applicants submit that the claimed method is enabled for any cell-containing fraction from blood or bone marrow (claim 47).

Step (b)

The method of claim 47 or 48 does not comprise the step (b). As explained above, the average expression of the genes in healthy human subjects has to be determined only once. Once the value is obtained, it can be used as a limit for the evaluation whether an elevated expression of at least one of the genes in the cell-containing fraction as compared to its expression in the further cell-containing fraction indicates the presence if disseminated cancer cells in the body fluid. Claim 47 states that this is the case if the ratio of gene expression from the cell-containing fraction to the further cell-containing fraction is higher than the average ratio of its expression in subjects not having cancer.

Step (c)

As there is no method "step (b)" in claim 48 or 49, there is no method step (c), either.

Step (d)

Step (d) requires the isolation of mRNA from the cell-containing fraction. Claim 48 is not limited to mRNA analysis but encompasses any means for determining the expression of the genes at stake. Applicants note that the invention is based on the finding that the disseminated cancer cells over-express genes involved in ant oxidative protection. That means that it is not only the mRNA but also the corresponding protein whose level of expression is elevated. The present specification therefore teaches that it is possible in principle to employ all the methods known to be suitable for quantifying proteins and nucleic acids from the fields of the protein analysis and nucleic acid analysis (see the present specification on page 12, line 35 to page 13, line 2). Applicants therefore submit that the present specification enables more than mRNA analysis. However, Applicants note that that claim 48 is specifically directed to mRNA analysis.

Step (e)

Step (e) requires measuring mRNA by reverse transcription and PCR. Applicants submit that the person of average skill in the art is acquainted with numerous methods for specifically quantifying nucleic acids (see, for instance, page 14, lines 22-33, and page 20, line 25 to page 27, line 38). The method of claim 47 or 48 does not need RT-PCR for enabled.

Step (e) further requires that specific primers are used for measuring the expression level of the genes. Claim 47 recites that the genes are those, which encode an mRNA that is capable of being amplified by the primer. Although the

method of claim 47 is not limited to the use of the primers, it nonetheless requires that the genes are capable of being amplified using the primers. Thus, irrespective of the gene at stake and its actual sequence, the person of average skill in the art would be in a position to determine its expression using the primers. Thus, the method of claim 47 is fully enabled by the specification to the present application.

Moreover, applicants note that claim 47 unambiguously defines the genes at stake by referring to the amino acid sequence of the proteins they encode.

Step (f)

In step (f), the expression level of GAPDH is determined. It is for practical reasons that the expression level is usually expressed as normalized cell equivalence (CEQ). To this end, the expression of a so-called housekeeping gene (e.g. GAPDH) is determined and the expression value obtained for the gene at stake is divided by the value obtained for the housekeeping gene, so that the value obtained for the expression of the gene at stake does not depend on the number of cells in the sample subjected to measurement. Such normalization is standard and known to the skilled artisan. Therefore, neither claims 47 nor 48 recite such a step.

Step (g)

As there is no step (f) in claim 47 and 48, there is no step (g), either.

Step (h)

As explained above for step (b), the average expression ratio is

determined once and the resulting limit for expression can be used without the need of repeating the determination. The method of claim 47 or 48 therefore does not recite such a step.

Step (i)

Applicants respectfully submit that the step of comparing the expression for each of the at least two genes in the cell-containing fraction with its expression in the further cell-containing fraction (claim 47) corresponds to step (i).

Step (j)

Claim 47 recites that an elevated expression of at least one of the at least 2 genes in the cell-containing fraction as compared to its expression in the further cell-containing fraction indicates the presence of disseminated cancer cells in the body fluid if the ratio of its expression from the cell-containing fraction to the further cell-containing fraction is higher than the average ratio of its expression in subjects not having cancer. Applicants respectfully submit that this step corresponds to the requirement that an expression ratio higher than the limit for at least one of MNSOD, TXNRD1 or GPX1 indicates that the disseminated cancer cells are present in the blood test sample.

If one compares the step (a) to (j) with claims 48 and 49, a similar picture emerges. However, the method of claims 48 and 49 does not require the enrichment of cancer cells when providing the cell-containing fraction, the provision of a further cell-containing fraction and the comparison of the gene expression in the cell-containing fraction with the further cell-containing fraction.

Applicants respectfully submit that the comparison with the average expression of the genes at stake in healthy patients is sufficient.

The Advisory Action of April 24, 2009 contends that Seven et al. provide evidence of the unpredictable nature of the invention. Applicants respectfully disagree.

Seven et al. assessed plasma lipid peroxidation and erythrocyte antioxidant status markers including CuZn superoxide dismutase (SOD1) and glutathione peroxidase in patients with laryngeal carcinoma. They found lipid peroxidation was significantly higher in the carcinoma group. However, none of the antioxidant markers, with the exception of vitamin E, show significant differences were noted on comparison of the carcinoma group and control group.

Seven et al. also report that their literature survey related to antioxidant response and cancer revealed that the findings are controversial. In this regard, Seven et al. refer to reports that assessed antioxidant levels in serum, erthrocytes or tumor tissue. They further state that the controversial findings in literature may arise from the type of cancer/tissue studied. The adaptive anti-oxidant response against oxidant stress is thought to be tissue-specific.

However, the claimed method is drawn to the detection of disseminated cancer cells, not to be assessment of antioxidant markers in plasma, erythrocytes of tumor tissue.

Although disseminated cancer cells are usually derived from a solid tumor, they are regarded as an independent tumor entity once circulating in the body fluid of an individual.

In the working examples, it is shown that irrespective of the tumor tissue from which they are derived the disseminated cancer cells over-express the genes at stake.

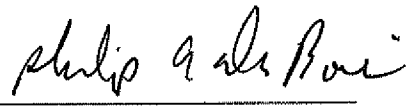
Thus, even if it were assumed that the level of antioxidants may depend on the tissue studied, the present specification provides evidence that disseminated cancer cells in general are characterized by an elevated expression of at least one of the genes at stakes.

Applicants note with appreciation the indication of the subject matter that the Examiner considers enabled by the present disclosure (see Official Action of January 5, 2009). However, applicants note that steps (a) to (j) closely reflect the subject matter set forth in the examples of the present specification. In this regard, the Examiner's attention is respectfully directed to MPEP § 2164.02, which provides that compliance with the enablement requirement does not turn on whether an example is disclosed. In fact, examples are not even required so long as the invention is otherwise disclosed in such a manner that one skilled in the art would have been able to practice the invention without an undue amount of experimentation. In re Borkowski, 422 F.2d 904, 908, 164 USPQ 642, 645 (CCPA 1970). Applicants respectfully submit that one skilled in the art would have been able to practice the invention set forth in the claims for the reasons discussed above and in the Amendment of April 13, 2009. In this regard, Applicants respectfully submit that requiring the claims to recite steps (a) to (j) unduly limits the claims and is improper as a matter of law. Applicants respectfully that all of the pending claims satisfy the enablement requirement and

respectfully request that the rejection be withdrawn.

In view of the present Amendment and Amendment of April 13, 2009, Applicants respectfully submit that the present application is in condition for allowance at the time of the next Official Action.

Respectfully submitted,

By: 
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